STAIN TECHNOLOGY

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PROGRESS IN THE STANDARDIZATION OF STAINS

RECENT ACTIVITIES OF THE STAIN COMMISSION

History of the Commission. The early history of the Stain Commission was discussed in the first issue of STAIN TECHNOLOGY,* where it is available to anyone interested. The only reason for summarizing it now is that the Commission was recently reorganized, and some understanding of its history is necessary to put its present status into the proper background.

When the first World War ended it was found that the German stains upon which biologists had previously been dependent were no longer available in America; and laboratory workers who had been forced to use products of American manufacture in Army camps and hospitals during the war reported that they had not been able to obtain the same results as they were used to in the past. To put it mildly, their remarks about American stains were not exactly complimentary. Most of these complaints came from bacteriologists or pathologists, and many of them reached the ear of Col. A. P. Hitchens, who was at that time Secretary-Treasurer of the Society of American Bacteriologists. He referred the matter for investigation to that Society's Committee on Bacteriological Technic, of which the writer of this note happened to be Chairman.

The original idea of the investigation was to confine it to bacteriological stains and to have it conducted by members of the Society. It was soon realized that other fields of biology were equally involved, and the problem was taken up by the National Research Council. The latter organization, in November, 1921, appointed a Committee on Standardization of Biological Stains, which functioned a year and then turned over its work to a newly formed organization known as the Commission on Standardization of Biological Stains. The Commission, as originally constituted, consisted primarily of the collaborators who had been appointed by the original committee to assist in its investigations; the Executive Committee of the Commission, on the other hand, was composed of representatives appointed by several national scientific organizations covering fields in which biological stains are em-

ployed. Originally there were five such society representatives, but their number was later increased to eight, to include other fields of science. Essentially this form of organization remained unchanged until early in 1944.

Finances. The original investigations, as planned under the Research Council, were modest in scope, and called for no high finance to organize them. For the small amount needed to hire necessary clerical work, the coöperation of the Chemical Foundation was secured. Its president, the late Mr. F. P. Garvan, proved very much interested in the work, and as the activities of the Commission expanded, his organization contributed larger and larger annual sums, so that the Commission was able to hire its own employees to do much of the testing, instead of having to depend wholly on voluntary collaboration. In all, the Foundation must have contributed about $100,000 to this work.

Meanwhile the Commission had slowly been building up an income of its own, partly from the sale of publications but largely from fees charged to manufacturers of biological stains. During the period from 1931 to 1938 the earned income was increasing and the contributions from the Chemical Foundation decreasing. Early in 1939 the latter stopped entirely, because of the expiration of patents on which the Foundation had been dependent. Fortunately this year was also the one in which the earned income of the Stain Commission became practically enough to balance its budget.

Reorganization. Largely because of this changed financial status, it seemed that the organization should be incorporated. Steps looking toward this incorporation were taken during 1942 and 1943. The actual incorporation took effect on Feb. 21, 1944, as explained in the footnote to the recently published bylaws of the corporation. This incorporation took effect under the terms of the charter granted by the University of the State of New York.

According to this charter the organization now becomes known as the Biological Stain Commission, Inc., which is organized as a non-profit corporation to take over the duties and assets of the Commission on Standardization of Biological Stains. The members of the Executive Committee of the defunct organization become trustees of the corporation with the addition of three new members, bringing the total number of members of the Board of Trustees up to eleven. Between meetings of the Board, the affairs of the Commission are managed by an Executive Committee of three. The officers consist of President, Vice-President, Secretary and Treasurer.

Membership qualifications. At the time of meeting for reorganiza-

tion, two important committees were appointed. One of them, of which Dr. R. D. Lillie is chairman, is a Membership Committee. The object of this committee is to establish qualifications for membership. As already explained, the original membership of the Commission consisted of those collaborators who had taken part in the early investigations. There were about 60 of them. As this original membership decreased, invitations were extended informally to others who were known to be interested in the work, and in that way the membership was maintained at approximately its original figure. It was felt, however, that the membership of the new organization should be put on a more satisfactory basis. It was agreed that its charter members should be those of the old Commission who were still active; it was also agreed that others should be invited, and the Membership Committee was appointed to determine qualifications and to pass upon new members that would be proposed. After a meeting, this committee recommended that members should be admitted only on invitation, that each new member should be proposed by one of the trustees, seconded by another trustee, and then a statement of his qualifications, accompanied by a list of publications in regard to dyes, staining, or microtechnic in general, should be submitted to the Membership Committee for action. This recommendation was adopted.

Plans for Research. The other committee appointed may well prove more important. It was a Research Committee, under the chairmanship of Dr. W. F. Windle. The object of this committee is to determine desirable fields for research along the lines of interest to the Commission and to decide how to organize work in those fields. This committee has been carrying on considerable correspondence since the meeting and is working on a report to submit to the Board of Trustees. It is hoped that future issues of STAIN TECHNOLOGY may have further statements as to the activities to be stimulated by this committee.

Publications. The demand for the book BIOLOGICAL STAINS has proved so great, since the United States entered the war, that the fourth edition is now out of print. Work on the fifth edition is in progress; but as more changes are contemplated than have been made in any recent edition, it is taking considerable time to get the revised manuscript in final shape. This fact, together with the delays in printing that are to be expected under present conditions, makes the date of publication of the new edition quite uncertain.

Meanwhile the Commission is sponsoring the publication of a

new manual, known as Staining Procedures (by H. J. Conn and Mary A. Darrow). This is a loose-leaf publication, designed to contain methods that are employed by members of the Commission, which is being issued, leaflet by leaflet, as they are completed. At the present time seven leaflets have been published, and two more (which will probably complete the series) are almost ready. (See advertising pages for more information.) After the entire series has been completed it is planned to keep the publication up to date by occasionally revising leaflets or issuing supplementary pages to include additional methods. In this way it is hoped to keep Staining Procedures a living publication that is not continually getting behind the times.

The Future. It is hoped that the new form of organization will allow greater developments in the future than the past has seen. Just what they will be, it is still hard to say; but there is no question but that there is a field for research and for standardization activities in regard to other stains and related products that have not yet been covered by the work of the Commission. Suggestions from anyone interested will be welcome.—H. J. Conn.

PREPARING SPECIMENS OF BONE AND TEETH FOR CUTTING BY THE PARAFFIN METHOD

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ABSTRACT.—**A method of preparing bone or teeth for sectioning is described which involves the following steps: 48 hr. in 1:10 formalin; 24 hr. in 70% alcohol; decalcification for several days in 10% HNO_3; rinsing and transferring to 2% potassium alum for 12 hr.; rinsing and treating with 5% $NaHCO_3$ (or Li_2CO_3) for 24 hr.; washing for 12–24 hr.; then passing through ascending grades of alcohol to xylene. In the case of developing teeth, a slightly different procedure is recommended: fixation in Heidenhain's SUSA till hard tissue is decalcified; 24 hr. in 96% alcohol (with three changes); 24 hr. in absolute alcohol (with one change); clearing in xylene or chloroform, and embedding in paraffin.**

The demonstration of the minute structure of bone and teeth, just as is the case with other tissues of the body, requires quite thin sections, which will still possess good staining qualities, despite the fact that the tissues have been decalcified.

Because of the many difficulties associated with the making of paraffin sections of bone and teeth, until recently the cutting of these tissues has customarily been carried out by use of the freezing or celloidin methods.

MATURE BONES AND TEETH

The present communication gives an account of a method by which specimens of bone and teeth can be brought through to paraffin, thus allowing the cutting of thin sections with a fair degree of certainty.

It is not claimed that the method is new in its entirety, but it *is* claimed that if the processes be carried through as described, reliable results should be obtained.

Most of the specimens prepared were stained by the Azan or Picro-Mallory (Lendrum and McFarlane, 1940) technics, with modifications where necessary. Excellent pictures of adult and developing teeth have been obtained, the detail and contrast being far superior to that given by the ordinary hematoxylin and eosin methods. In addition, it has been found that even the somewhat capricious Schmorl's method gives more certain results with a

greater degree of brilliance in staining than are obtained with the freezing and celloidin methods. One of the most important features of the method is that there is little or no shrinkage of the soft tissues away from the hard tissues; for example, the soft and delicate tissue of the pulp of the tooth, with its lining border of odontoblasts lies in perfect apposition to the dentine, giving an undistorted picture of their relationship.

Method: The specimens of bone and teeth are first of all sawn to suitable dimensions before placing them in the fixative. Specimens which will show bone and teeth at the same time are obtained by sawing through the lower jaw of a cat or kitten in the spaces between the teeth and placing the pieces in the fixative. They are then treated as follows:

(1) Fix for at least 48 hours in 1:10 formalin.
(2) 70% alcohol 24 hours.
(3) Transfer to 10% HNO_3. Leave till thoroughly decalcified, changing the acid every second day. The time of decalcification will of course depend on the size of the piece of tissue; always trim away as much excess tissue as possible.
(4) Rinse in one or two changes of water, a few seconds only, and transfer to 2% potassium alum; leave for 12 hours.
(5) Again rinse in water, transfer to 5% $NaHCO_3$ or Li_2CO_3 (half saturated) for 24 hours.
(6) Wash in running water using a washing bobber (Gairns, 1942) for 12–24 hours. The tissues are now ready for carrying through to paraffin. The time-table given here will be found to be most convenient for the routine day of the laboratory
(7) 10% alcohol—9 a.m. to 12 noon.
(8) 20% alcohol—12 noon to 5 p.m.
(9) 40% alcohol—overnight.
(10) 60% alcohol—9 a.m. to 12 noon.
(11) 80% alcohol—12 noon to 5 p.m.
N.B. It is an advantage to add to these alcohols phenol up to 6% (Lendrum, 1935).
(12) Absolute alcohol, overnight until 10.30 a.m., changing the alcohol at 9 a.m.
(13) Xylene, 10.30 a.m. to 12 noon.
(14) 52°C. paraffin, 12 noon to 2.30 p.m.; then 60°C. paraffin until 4.30 p.m., changing once.
(15) Embed and cool.

Any of the usual clearing agents may be used instead of xylene. Benzene, butyl alcohol, cedarwood oil, and aniline oil, have been

tried without any noticeable difference. Xylene seems to require a shorter time for clearing which is perhaps of some slight advantage. After the paraffin block has been trimmed and fixed to the nosepiece of the microtome, it should be allowed to stand in very cold water for about 10 minutes. This makes an astonishing difference to the cutting of the sections.

Developing Teeth

In the case of developing teeth, a different procedure is required. Undoubtedly the finest fixative for jaws of young animals is Heidenhain's Susa, formula:

Saturated aqueous $HgCl_2$	50 ml.
Formalin	20 ml.
Glacial acetic acid	4 ml.
Distilled water	30 ml.
Trichloracetic acid	2 g.

It should be noted that Susa fixes and decalcifies at the same time. It is perhaps a little slow in its decalcifying action but the time involved is well worth while as the end results give eminent satisfaction. It is also one of the best fixatives to use if aniline dyes are to be employed for staining.

Method:

(1) If embryo jaws are used, immerse in Susa till the hard tissue is decalcified. If the specimens are taken from animals after birth, extra trichloracetic acid (up to about 10%) can be added to the Susa solution. This accelerates the decalcification and at the same time is not too drastic.
(2) Transfer direct to 96% alcohol; change three times in 48 hours.
(3) Absolute alcohol 24 hours; change once.
(4) Xylene or chloroform until clear.
(5) Embed in paraffin, 4 hours at 52°C.

Note:—Since Susa contains $HgCl_2$, the sections must of course be treated with iodine and thiosulfate before staining.

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LA TECHNIQUE DE TANNIN-FER

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Abstract.—**The author points out that he should be accredited for originating the tannic-acid-iron method for staining tissues. Reference to *Comp. Rend. Soc. Biol.*, volume 83, 1920, shows that Salazar introduced the method at that time. Several writers in recent years have failed to acknowledge this work.**

The author has used this technic in studies of the Golgi apparatus in the ovary and the thyroid gland. In the field of hematology, he has combined the tannic-acid-iron method with Giemsa stain and claims that his method allows a sharper differentiation between agranulocytes and granulocytes, as well as a satisfactory revision of the Arneth classification of neutrophils.

More recently the author has devised several new modifications of the tannic-acid-iron technic which he has not yet published. He feels that these modifications should be a distinct aid to cytology and histology. He concludes: "One might say that the tannic acid and iron reaction has already created a new chapter in histological technic, a chapter which is not yet closed."

La méthode au tannin-fer a été introduite par nous dans la technique en 1920 (1), ce que plusieures auteurs, Rösler, Zimmermann, Korody, Härtel et encore récemment Sharman, semblent ignorer (2).

Nous avons montré dans une série de travaux publiés après 1920 les résultats qu'on peut obtenir en général avec cette réaction et particuliènement ceux qu'on obtient dans l'ovaire et dans l'analyse du complexus de Golgi, etc., tandis que mon élève Bacelar étudia les résultats de la même technique dans l'étude de la thyroide (3–18).

Nous avons essayé de débrouiller la question du mordançage tannique, et nous avons surtout mis en relief que la réaction tanno-férrique est très spécialisée: elle est surtout caractérisée par sa propriété de ne pas colorer les graisses et les lipides et de, dans la classe des protéiques, ne colorer que certains types de substances.

Nous avons d'un autre côté montré que la réaction tanno-férrique peut servir de réaction matrice à plusieurs méthodes, que nous avons divisées en deux groupes: combinaisons prè-tanniques et post-tanniques.

Dans les prémières le tannin actue comme différentiateur après le colorant et comme mordant pour le fer; dans les deuxièmes, le

système tanno-férrique actue comme mordant complexe. Nous avons alors décrit comme principal combinaison pre-tannique la méthode de la safranine-tannin-fer, et comme types de combinaison post-tannique l'hématoxyline tanno-férrique et le tannin-fer-Giemsa (19).

À l'aide de ces techniques nous avons fait l'étude de plusieurs questions, en particulier de la zone de Golgi et de l'appareil para-Golgi, découvert en 1924 (4) avec la technique du tannin-fer simple. Nous avons également à l'aide du tannin-fer-Giemsa établi plusieurs faits nouveaux en hématologie, tels que la réaction cyanotannophile, les "granula tannophila", et d'autres faits qui nous ont permis de donner une autre classification des éléments du sang (pachy-neutrophiles, meta-neutrophiles, lepto-neutrophiles), en rapport avec l'évolution des éléments. Ces mêmes faits nous ont permis d'établir une différentiation plus nette entre les familles lymphoïde et granulocytique (20).

Ils nous ont permis de compléter le tableau cytologique des granulocytes, en bases nouvelles, de revoir, à partir de là, les idées d'Arneth et d'autres auteurs sur les formules neutrocytaires, l'équilibre neutrocytaire, etc. Ces mêmes faits nous ont permis encore de revoir certaines questions fondamentales de l'hématologie comparée (5).

La réaction du tannin-fer simple nous a permis encore en collaboration avec Ad. Estrada, de découvrir l'énigmatique réaction-choc des globules rouges et de l'hémoglobine (15).

La réaction tanno-férrique nous à conduit au concept de "tannophilie", "de substances tannophiles", accepté ensuite par les auteurs (Wallraff, Hruby, Stosiek, Tonutti, C. da Costa, Tavares de Sousa etc.), analogue à ceux de sidérophilie, argentophilie, etc., concept dont l'utilization a donné déjà des résultats positifs.

De la technique du tannin-fer dérivent encore les réactions du tannin-fer-acéto-ammoniacale et tannin-fer-transfert, introduites également par nous dans la cytologie et qui sont des réactions spécifiques du noyau (21).

La technique du tannin-fer et les méthodes qui en dérivent est caractérisée, d'après Beckert, "par sa simplicité, sûreté, vitesse et netteté de coloration" (22).

Ces méthodes ont determiné, d'après Wallraff (23), un certain renouvellement dans l'étude de plusieures questions cytologiques de l'hypophyse, de la thyroide, de l'estomac, du pancréas, etc. (23–28).

Ils ont permis également une analyse plus poussée de la zone et du complexus de Golgi, en particulier la découverte de l'appareil para-Golgi, le composant protéique du complexus (29).

Sous le point de vue de la coloration du conjonctif, d'après Tonutti, "die Méthode eignet sich im übrigen ausgezeichnet zur Wiedergabe der Präparate im Lichtbild und was mir schliesslich als das Allerwichtigeste erscheint, die Darstellung der Bindegewebsubstanzen ist unabhängig von Differenzierungseinflüssen, und damit objektiv. Ihre Anwendung bei vergleichenden Untersuchungen ist daher besonders zu empfehlen" (28).

L'analyse du conjonctif à l'aide des techniques au tannin-fer n'a pas encore été faite; du reste il y a encore un vaste champ de recherches à réaliser avec l'application de ces méthodes au tissu nerveux et dans plusieurs organes.

Nous avons montré déjà que ces méthodes peuvent être utilisées en cytologie végétale, et nous avons déjà montré, à l'aide de ces moyens, le non fondé de la théorie du vacuome (30) et de certaines théories sur les rapports entre le plastidome et le chondriome (32).

Tout récemment nous avons encore essayé, avec des resultats positifs, d'autres techniques au tannin-fer, conduisant à des resultats nouveaux.

Ces techniques, encore non publiées, ont pour base un mordançage tannique alterné et successif, jusqu'à obtenir une réaction noire intégrale suivie ou non de différentiation. Cette technique, que nous appellons tannin-fer-II, est un nouveau moyen d'analyse important, comme nous le montrerons sous peu.

Nous avons également essayé avec des résultats positifs, des combinaisons pre- et post-tanniques accouplées, à l'aide desquels ont peut réaliser de nouvelles méthodes polychromiques.

En resumé, la technique du tannin-fer, a permis déjà d'établir un ensemble considérable de faits, concepts et théories nouvelles en histologie et cytologie. Elle a été l'origine, d'un autre côté, de toute une série, déjà assez nombreuse, de nouvelles méthodes histologiques et ctyoloqigues, telles que la méthode de Wallraff, le tannin-fer-Giemsa, l'hématoxyline tanno-férrique, la safranine-tannin-fer et plusieures autres. On peut dire que la réaction du tannin-fer a créé déjà un chapitre nouveau de la technique histologique, chapitre qui n'est pas encore clos et dont les ressources sont encore en grande partie à explorer (22, 23, 24, 25, 26, 27).

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THE RAPID PREPARATION OF CELLOIDIN SERIAL SECTIONS FOLLOWING INDIA INK INJECTIONS

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ABSTRACT.—**For the study of capillary penetration in the central nervous system of the chick embryo, following India ink injections, celloidin serial sections are superior to those prepared by the paraffin technic. The celloidin sections are arranged on a moist cigarette paper mat, which when filled is inverted and applied to a microscope slide so that the sections contact the glass surface. Subsequent to dehydration and clearing the sections are isolated on the slide by peeling off the cigarette paper. Forty-five minutes are required to prepare a slide of thirty sections from the time the block is trimmed until the cover slip is mounted with Clarite.**

If the details of capillary penetration into such structures as the central nervous system are to be studied following India ink injections of chick embryos, serial sections are needed. Paraffin serial sections are not adequate for this purpose since removal of the paraffin allows the carbon particles to escape from the capillaries. Celloidin sections are essential for restricting the carbon particles to the lumen of the blood vessels. However, the usual methods for preparing celloidin serial sections are much too tedious and time-consuming to be useful. Consequently a method for the rapid preparation of celloidin serial sections has been developed and is described below.

The embryos should be fixed in Zenker's formol as described by Guyer (1936) since such fixation may be followed by a wide variety of stains. After fixation and thorough washing in tap water, the embryos are stained *in toto* with 1.0% alum carmine for 24 hours. (Other useful stains include tincture of cochineal with $CaCl_2$ and and $AlCl_3$, iron cochineal, magnesium carmine, and hydrochloric carmine; see Lee, 1937, pp. 139–49, for details.) They are then rinsed in tap water, and are next stained for 24 hours in 0.5% orange G. The orange G has a solubility of 1.80% in water and .22% in alcohol which permits differentiation of the alum carmine during subsequent dehydration in alcohol without removal of all of the cytoplasmic stain. A quick celloidin infiltration, such as the one described by Guyer (1936) lends itself well to the preparation of specimens up to 96 hours of incubation. Older specimens require longer periods for complete infiltration. The knife holder is clamped to a seven-inch microtome blade at its distal end with reference to the

operator. Cigarette paper, cut the size of a microscope slide, serves as a transferring mat. It is placed on the end of the blade opposite the knife holder (Fig. 1). During the sectioning process the microtome blade is flooded with an excess of 80% alcohol containing some orange G. This stain must be present in the alcohol solution in order to prevent the complete diffusion of the orange G from the sections. Only enough of the stain is needed to give the alcohol an amber color; otherwise unsightly crystals will form on the sections. The meniscus of alcohol formed along the cutting edge of the microtome knife should be in contact with the mat. Any extreme excess of alcohol will spill off the blade at the end of the stroke so that it is almost impossible to use too much of this solution. As the sections are cut they are guided down the knife and onto the

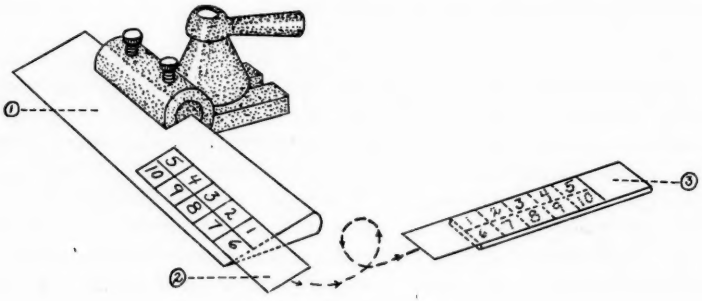

FIG. 1.

(1) Microtome blade. (2) Cigarette paper mat. (3) Slide.

cigarette paper with a No. 1 camel's-hair brush. Movement of the sections on the mat is made possible by directing the flow of alcohol, held in a No. 1 camel's-hair brush, under the section followed by gentle strokes for proper orientation. The sections are arranged in order on the paper from right to left (Fig. 1). Capillary action draws enough alcohol to the surface of the cigarette paper to keep the sections moist without allowing them to move from their position. The mat is raised higher on the blade when the last row of sections is being arranged to prevent any shifting of the sections due to movements of the alcohol meniscus. When the mat is filled with sections it is gently lifted from the blade with the left hand, inverted, and then applied to the slide (Fig. 1). When sectioning is discontinued the celloidin block should be covered with a cotton swab saturated in alcoholic orange G solution. While the mat is being lowered onto the

slide it should be guided with a pair of forceps held in the right hand. The margins of the cigarette paper and slide will accurately coincide if this procedure is followed. Care must also be taken to prevent air bubbles from forming between the glass surface and the sections. After the paper is oriented with respect to the slide there should be enough room on the right hand margin for the label. A blotter is then carefully pressed to the mat so that no sections are forced out from under the cigarette paper.

Dehydration is accomplished by applying 95% alcohol to the exposed surface of the mat, followed by blotting after sufficient time has elapsed for complete penetration. Three such rinses with alcohol followed by blotting are necessary to remove the excess orange G that has become included within the celloidin. Carbol-xylene is then applied to the mat and allowed to remain for 3 minutes. This

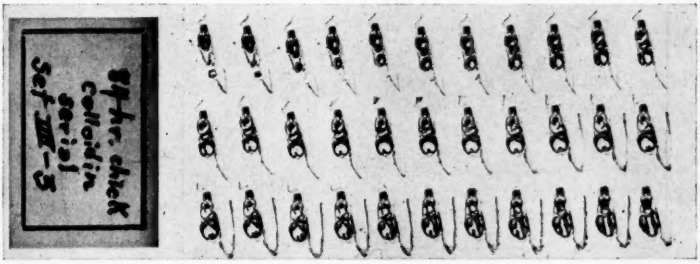

FIG. 2. Sample slide. × 1½.

period is usually sufficient to clear the sections; however, a longer application may be necessary on thicker specimens. After thorough blotting, the sections are left adhering to the slide by peeling off the cigarette paper. If the blotting has been complete there is no tendency for the sections to peel off with the mat. Thin Clarite is then added to the sections before they dry. Cover slips are applied, and are pressed tightly to the slides.

Once the block has been trimmed, a slide containing 30 serial sections, complete with cover slip, can be prepared in 45 minutes (Fig. 2). Although this procedure was developed for chick embryos, it can be readily applied to any animal or plant tissue. Moreover, if the staining is not done *in toto*, the sections can be covered with a thin film of celloidin after dehydration and stained like paraffin sections.

REFERENCES

GUYER, M. F. 1936. *Animal Micrology.* University of Chicago Press. See pp. 29 & 68.

LEE, A. B. 1937. *The Microtomist's Vade-Mecum.* Churchill, London. See pp. 139–49.

MODIFICATION OF PROCEDURE FOR DIFFERENTIATING THE TELIA OF *CRONARTIUM RIBICOLA* AND *C. OCCIDENTALE*

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Telia of the pine rusts, *Cronartium ribicola* A. Fisch. and *C. occidentale* Hedgc., Bethel, and Hunt, on the leaves of *Ribes* are morphologically indistinguishable. The ranges of these rusts now overlap in parts of the West and, since one is under quarantine regulation and the object of a large-scale control program while the other is of little consequence, a method of reliable differentiation is required. Acree and Goss[1] have worked out a microchemical method that has proven successful in distinguishing between the two species at the telial stage, but if large numbers of tests are being made a shorter schedule than that described by them is advantageous. Accordingly, attempts to work out a shorter schedule were made. The following procedure was found to give as satisfactory differentiation between the species of rust as the longer method when both schedules were compared on more than 184 specimens over a period of three seasons:

1. Under a dissecting microscope cut out a piece of *Ribes* leaf blade approximately 5 × 5 mm. on which grow mature ungerminated telia. Germinated or parasitized telia do not give a satisfactory reaction.

2. Place in watch glass and cover with 2 ml. N/10 HCl. Under the dissecting microscope tease out all air bubbles with a camel's hair brush so that all parts of the telia are in contact with the acid. Leave specimen, telium-side down, in acid 5 minutes.

3. Remove specimen, pass through distilled rinse water and wash by immersion for 10 minutes in distilled water, making two changes.

4. Remove specimen and blot on filter or neutral blotting paper. Place on slide under dissecting microscope and tease telia from leaf blade with a dull scalpel.

5. Allow telia to dry (1–3 minutes) before putting on drop of .001 *M* brom phenol blue with pH adjusted to 7.6. Place cover glass in

[1]Acree, Ruby J., and Goss, Warren H. 1937. A microchemical colorimetric pH procedure for differentiating the telia of *Cronartium ribicola* and *C. occidentale*. J. Agr. Res., 55, 347–52.

position and set aside 20 minutes before examining under low power of comparison or compound microscope.

Spore contents of *Cronartium ribicola* telia turn blue and those of *C. occidentale* green. If the color is not clear because of the large amount of brom phenol blue under the cover glass, place a drop of distilled water at one edge of the cover glass and with filter paper draw off the indicator from the other side.

It should be emphasized in examining the telia that the color to be observed is that of the spore contents and not that of the surrounding matrix. The latter is usually yellow and because of this *C. ribicola* telia sometimes appear greenish blue as a whole. Moreover, the bases of telia of *C. occidentale* are sometimes blue. This may be due to a failure during the acid bath to eliminate air bubbles around the telia where they arise from the leaf blade, so that the acid does not come in contact with them. Occasionally telia are encountered which stain only in the outer layers. Acree and Goss[2] have described the typical colors obtained with their schedule as being those found in Maerz and Paul[3], plate 34 L 7–12 for *C. ribicola* and for *C. occidentale* plate 30 L 6–12. In Ridgway[4], for *C. ribicola* they are plate VIII, light cerulean blue, cerulean blue, oxide blue, and Antwerp blue, and for *C. occidentale* plate VII light blue-green, Guinea green, and dark viridian green, and plate XVIII oriental and dark yellowish green. With the shortened schedule, shades of blue and green similar to, or approximating these colors, are obtained.

Where a number of specimens are to be examined time may be saved by running up to four samples concurrently, using the requisite number of additional watch glasses and wash bottles or beakers. In the intervals during the processing of one group of samples, others may be prepared for treatment.

[2]See footnote 1.

[3]Maerz, A., and Paul, M. R. 1930. A dictionary of color. 207 pp. Illus. New York.

[4]Ridgway, R. 1912. Color standards and color nomenclature. 43 pp. Illus. Washington, D. C.

NOTES ON TECHNIC

On the Cutting of Tough and Hard Tissues Embedded in Paraffin.

It has been known for some years that tissue embedded in paraffin has the power of taking up water, and that it is possible, after immersion in water of the tissue exposed by cutting into the paraffin block, to obtain a section from an otherwise uncuttable tissue. With animal tissues, however, this has certain disadvantages, and so Baker[1] was led to experiment with various other soaking fluids. The mixture of 9 parts 60% ethanol with 1 part glycerin that he found most satisfactory depends for its helpful action on the glycerin; the water and ethanol apparently act merely as accelerators of penetration. The main use of this fluid is stated by Baker to lie in the easier and finer cutting of brittle tissues such as liver, spleen or tissues containing much blood clot, a statement which our departmental experience very fully confirms. He then warns the reader that this mixture does not facilitate the cutting of tough tissues, of which he mentions as an example the formalin-fixed vagina of a bitch, but experience with the biopsy material in this hospital has shown that Baker's disclaimer is unduly cautious[2]. Tissues such as thyroid or scirrhous carcinoma of breast may well prove tough enough; and in friendly disagreement with Baker we have found that, if it prove difficult to obtain a decent section of such tissues, immersion of the block overnight in his mixture (or the similar mixture, marketed as Mollifex by the British Drug houses Ltd.) allows the cutting next morning of a thinner and better section. The value of this manoeuver is attested both by those who cut the sections and by those who must make the diagnoses.

More recently I have found that tough and hard human tissue is made even more amenable by soaking the opened paraffin block in a mixture of 9 parts of glycerin with 1 part of anilin. To achieve the best result may demand two or three days' soaking; this discolors the wax but seems to produce no other detrimental effect.

Thus to sum up our routine and the place of these adjuvants, paraffin blocks are mounted on hard wooden chucks and cut by the Cambridge rocking microtone. If the technician is dissatisfied with

[1]Baker, J. R. 1941. A fluid for softening tissues embedded in paraffin wax. J. Roy. Micr. Soc., **61**, 75–8.

[2]Lendrum, A. C. 1943. On the handling of small biopsy material. Brit. Med. J., 1943 ii, 644.

the section obtained, he transfers the block, still attached to the wooden chuck, to a dish containing a thin layer of Baker's fluid. The block lies open face down in the fluid till the following morning; it is then usually possible for him to produce the promised thinner and better section. If, however, the tissue be especially hard or if a section be wanted of demonstration quality from such difficult tissues, the block is soaked for two or three days in the anilin-glycerin mixture. ALAN C. LENDRUM, *Department of Pathology, The University and Western Infirmary, Glasgow, Scotland.*

STANDARDIZED TABLETS FOR THE RESAZURIN TEST.

While there has been a steadily increasing interest in the resazurin test for reduction in milk, one drawback to its widespread use among the smaller dairy plants has been the necessity for weighing out accurately small quantities of the dye powder in preparing a solution of the desired strength. This drawback has now been removed. A batch of tablets of resazurin, each of which will make up 200 ml. of working solution when dissolved in that amount of water, has been prepared by the National Aniline Division of the Allied Chemical and Dye Corporation. Samples of these submitted for testing by the Biological Stain Commission have been found quite satisfactory and this batch has been certified by the Commission. These tablets should now be available through the regular laboratory supply houses.—C. K. JOHNS, *Central Experimental Farm, Ottawa, Canada.*

LABORATORY HINTS
FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

DYES AND THEIR BIOLOGICAL USES

COPLEY, A. L., and WHITNEY, D. V. **The standardization and assay of heparin by the toluidine blue and azure A reactions. A correction.** *J. Lab. & Clin. Med.*, **29**, 117.

An error occurred in the previous paper (*J. Lab. & Clin. Med.*, **28**, 762. 1943 Stain Techn. **19**, 48). Throughout the paper, all the concentrations of toluidine blue and azure A, and all quantities given as gamma should be read one tenth of the reported values.—*John T. Myers.*

FINKELSTEIN, JACOB. **N^4-substituted sulfonamides.** *J. Amer. Chem. Soc.*, **66**, 407. 1944.

A series of chloracetyl, aminoacetyl and caproyl derivatives of sulfonamides was prepared and tested for their efficacy as chemotherapeutic agents. Experiments in mice suggested that these compounds have a low acute toxicity similar to sulfadiazine, sulfapyridine and sulfathiazole, and showed that many of the derivatives are very effective against such organisms as *Streptococcus*, pneumococcus (Type I), *Staphylococcus aureus* and *Salmonella schottmülleri*. The relative activity of these drugs was also tested against *Plasmodium lophurae* in Pekin ducklings. The caproyl compounds all showed about the same activity as the parent sulfonamides and the chloracetyl derivatives seemed uniformly less active.—*A. P. Bradshaw.*

FRAENKEL-CONRAT, H., and COOPER, M. **The use of dyes for the determination of acid and basic groups in proteins.** *J. Biol. Chem.*, **154**, 239. 1944.

The authors recommend the following reagents for the determination of the acid and basic groups in proteins. Dye solutions: 0.1% aqueous orange G (Coleman and Bell) and 0.2% safranin O (National Aniline); these percentages were computed from material dried to constant weight at 70° C, disregarding the "dye content" indicated on label. Buffers: for pH 2.2, 980 ml. 0.1 *M* citric acid and 20 ml. 0.2 *M* Na_2HPO_4; for pH 11.5, 250 ml. 0.2 *M* Na_2HPO_4 and 200 ml. 0.1 *N* NaOH, water to make 1000 ml.

The method recommended is: To each of four 15 ml. test-tubes suitable for use in angle head centrifuge are added 5 mg. of the protein, 1 ml. of the pH 2.2 buffer, increasing amounts (1, 2, 3 or 4 ml.) of 0.1% orange G and two glass beads. If the protein dissolves in the buffer, it will be precipitated by the dye. The suspensions are shaken mechanically for 20–24 hours. They are centrifuged and aliquots of supernatant solution are diluted 100-fold. Color intensities are determined by means of a photoelectric colorimeter (Klett-Summerson) with a blue filter (Corning No. 038 amd Pyrex No. 554). Dye concentrations are read from a standard curve prepared from data obtained with appropriate dilutions of orange G stock solution. The dye bound by the protein is determined by subtracting the excess found in the supernatant fluid from the amount added. The same method is used with the alkaline buffer and the safranin solution. Details of the results obtained by this method using various proteins are given in tabular form and are discussed.—*J. E. Kindred.*

GILMAN, HENRY, and SHIRLEY, DAVID A. **Some derivatives of phenothiazine.** *J. Amer Chem. Soc.*, **66**, 888. 1944.

Because earlier studies have shown certain phenothiazine derivatives (e.g. methylene blue) to have antimalarial activity the authors prepared some appropriately substituted, non-oxidized phenothiazines for testing in avian malaria.

It has been shown that phenothiazine and some of its simple derivatives have a low toxicity for animals, and that this toxicity is further reduced by substitution in the 10- position. For this reason, some 10-phenylphenothiazine derivatives were prepared. The 10-(4′-γ-diethylaminopropylamino)-phenylphenothiazine was the only one of these compounds which showed any activity, and this was of a doubtful nature.—*A. P. Bradshaw.*

GILMAN, HENRY, and SPATZ, SYDNEY M. **Some quinolines patterned as "open models" of atabrine.** *J. Amer. Chem. Soc.*, **66**, 621. 1944.

In connection with studies attempted on the correlations of constitution with antimalarial action, four compounds having essentially the same functional groups as atabrine but having a more open molecule were prepared. Antimalarial activity in avian malaria was shown by both 6-methoxy-2-(3′-chlorophenyl)-4-[(α-methyl-δ-diethylaminobutyl)-amino]-quinoline and the isomeric (4′-chlorophenyl) compound, thus showing that the position of the chlorine in this part of the molecule did not affect the activity. The presence of the chlorine was also shown not to be essential for activity, for 6-methoxy-2-(phenyl)-4-[(α-methyl-δ-diethylaminobutyl)-amino]-quinoline is also active. However the position of the methoxy group (in the chlorine free type) is important, because 2-(2′-methoxyphenyl)-4-[(α-methyl-δ-diethylaminobutyl)-amino]-quinoline, unlike the 6-methoxy isomer, is inactive.—*A. P. Bradshaw.*

KLOTZ, IRVING M. **The mode of action of sulfonamides.** *J. Amer. Chem. Soc.*, **66**, 459. 1944.

The inhibition of bacterial growth by sulfonamides may be accounted for quantitatively by assuming that the action is due to a reversible combination between the basic form of the drug and the neutral form of the protein, and that the law of mass action is applicable. Equations may be derived which relate drug potency to the acid ionization constant of the sulfonamide and to the pH of the solution.

The reversal of the sulfonamide bacteriostasis by addition of *p*-aminobenzoic acid may be considered from the same point of view. Expressions may be obtained which account for variations in the ratio of sulfonamide to *p*-aminobenzoic acid from drug to drug and from one pH to another.—*Author's summary.*

MUELLER, ALBERT C. and HAMILTON, CLIFF S. **Some derivatives of 7-methoxy- and 10-methoxybenzoquinoline.** *J. Amer. Chem. Soc.*, **66**, 860. 1944.

The synthesis of two series of methoxybenzo(f)quinolines with a basic substituent in the 1- position has been undertaken with the hope that the presence of the methoxyl would increase the antimalarial activity of this type of compound. Pharmocological testing of a number of these derivatives is in progress.—*A. P. Bradshaw.*

PETERSON, OSLER L. **Therapeutic effects of forbisen and of toluidine blue on experimental typhus.** *Proc. Soc. Exp. Biol. & Med.*, **55**, 155–7. 1944.

Mice infected with yolk sac strains of typhus were protected by toluidine blue used in concentrations of 0.25 to 0.75% in the diet, or 5 to 20 mg. per day. Forbisen even at a level of 2.5% was less efficacious. Infected cotton rats were not protected by these substances.—*M. S. Marshall.*

ANIMAL MICROTECHNIC

BALLANTYNE, E. N. **A staining method for frozen sections.** *Canad. J. Med. Techn.*, **2**, 65–7. 1940.

The following method is sufficiently rapid for quick diagnosis, and yields satisfactory permanent preparations: Fix tissue in 10% neutral formol, containing 0.85% NaCl, by heating solution to boiling. Wash in tap water. Place on freezing microtome in a drop of dextrin solution. Freeze, cut, and place sections in distilled water. Wash section in distilled water. Stain 10 sec. in 0.1% aqueous phloxine. Wash in distilled water. Stain 10 sec. in 0.5%

aqueous toluidine blue. Transfer to 50% ethyl alcohol, move about quickly to remove loose stain. Place in 80% alcohol for a few seconds. Float onto slide in distilled water. Drain off water and cover section with drop of 50% alcohol. Blot with clean white blotting paper. Drop 80% alcohol on the section, drain off excess. Drop 95% alcohol on section, drain off excess. Dry slide edges carefully. Cover section with drop of euparol mounting medium and apply cover slip.—*C. Randall.*

BODIAN, DAVID, and MELLORS, ROBERT C. **Phosphatase activity in chromatolytic nerve cells.** *Proc. Soc. Exp. Biol. & Med.*, **55**, 243–5. 1944.

The authors found chromatolysis to be indicated by degradation of chromophilic material, a nucleoprotein of the ribose type, found in nerve cells. Increase in acid phosphatase activity was proportional to the degree of chromatolysis. Phosphatase activity was demonstrated as areas in sections darkened due to lead sulfide. These sections were prepared from spinal cord, soaked in a phosphate ester and lead nitrate. Inorganic phosphate, released by enzyme activity, was precipitated by lead nitrate, and lead sulfide was formed by treatment with ammonium sulfide.—*M. S. Marshall.*

FORBES, J. **Glycerine jelly mounting medium for frog eggs and early embryos.** *Trans. Amer. Micr. Soc.*, **62**, 325–6. 1943.

Frog eggs or embryos in alcohol, water, or formalin may be mounted directly in glycerin jelly for use by students. The jelly is prepared as follows: soak 28 g. of gelatin in 120 ml. distilled water until soft; add 140 ml. glycerin; heat gently until gelatin is dissolved; add 2 ml. melted phenol; filter twice through glass wool in a heated funnel; collect the second filtrate in an agate-ware pie pan; brush bubbles to one side; allow to solidify; cut into 1 inch squares; and wrap in No. 72 waxed powder paper. To use for mounting, heat a square very gently on the slide by superimposing it on another slide which has been heated, then put the egg in place and cover it.— *Virgene Kavanagh.*

HUGHES, R. F. **Laboratory hints.** *Canad. J. Med. Techn.*, **3**, 25–6. 1940.

The following technic is excellent for differential staining of eosinophilic granules in nasal discharges: Tease out secretion on slide and dry gently over flame. Stain with 0.5% alcoholic eosin, 1 min. Add enough distilled water to take up staining solution and let stand 1 min. Drain off and wash with distilled water. Flood with 95% ethyl alcohol. Drain off the alcohol, and immediately stain 1 min. with 0.1% alcoholic methylene blue. Flood slide with distilled water and let stand 2 min. Remove excess stain with distilled water. Cover slide with ethyl alcohol. Drain off the alcohol, and examine. Restain with methylene blue if neutrophiles do not stain well. If blue is too intense, flood slide with weak acid solution (1 drop 1% HCl in 1 ounce distilled water); quickly pour off, flood slide with distilled water, and finally with ethyl alcohol.—*C. Randall.*

KRAJIAN, ARAM A. **Elastic fibre stains.** *Canad. J. Med. Techn.*, **3**, 207. 1941.

The author proposes the following technic: Fix tissues in 10% formaldehyde 24 hr. or longer. Cut frozen sections about 10 μ. Place in 2% aqueous $AlCl_3$ for 5 min. Wash thoroughly in tap water. Place for 10 min. in the following stain: 4% aqueous Congo red, 8 ml.; glycerin (C.P.), 2 ml. Wash quickly in tap water. Rotate section for 10 sec. in 1% aqueous KI. Wash thoroughly in tap water. Place for 5–10 min. in the following stain: resorcinol, 3 g.; aniline blue, 1.5 g.; orange G, 2.5 g.; phosphomolybdic acid, 1.0 g.; distilled water, 100 ml. Wash in tap water. Float section onto slide and blot with filter paper. Dehydrate 2 min. in 3 changes of absolute alcohol (from dropping bottle). Clear for 2 min. in oil origanum. Clear in pure xylene. Mount in gum dammar.

This method gives a well-differentiated preparation in which elastic fibers appear bright red; fibrin and connective tissue, dark blue; red blood cells, orange yellow.—*C. Randall.*

KUPPERMAN, H. S., and NOBACK, C. R. **A rapid iron hematoxylin tissue stain for laboratory use.** *Science*, **98**, 591–2. 1943.

A modification of the standard Heidenhain iron hematoxylin stain is described in which the processes of fixation and mordanting are executed simultaneously in a solution prepared by using 1.5 g. ferric ammonium sulphate per 100 ml. of Bouin's fluid, the time for fixation and mordanting for small (5 to 6 mm. cube) pieces of tissue being the same as usual. After removal of the tissue from the fixative-mordant, it is stained with 0.5% hematoxylin, prepared for mounting by the usual procedure, using either alcohol or dioxane. When sections are passed through alcohol to the stain, the process must be rapid to avoid washing out the mordant. Sections are overstained in 3 to 5 minutes and destaining may be carried out as desired in 0.1% aqueous or 35% alcoholic HCl, followed by an alkaline wash in a solution prepared by adding several ml. of saturated aqueous $LiCO_3$ or 1% $NaHCO_3$ to 100 ml. of either water or 35% alcohol.

Mordanting just prior to the staining is unnecessary. This modification of the usual procedure makes a rapid and simple stain for routine laboratory use with the resulting stain comparable to that observed after use of the accepted and lengthy Heidenhain iron hematoxylin method.—*T. M. McMillion.*

LAWS, S. G. **A method of staining blood films.** *Canad. J. Med. Techn.*, **3**, 63. 1941.

The following method for staining occasional blood films employs stock solutions of 1% aqueous azure II and 1% aqueous eosin that may be kept indefinitely: Fix blood films. Mix 1 ml. of diluted Azure II (stock solution diluted 10 times) with 9 ml. dilute eosin (3 ml. stock solution plus 500 ml. distilled water). Pour a little on slide and let stand 20–30 min. Wash in distilled water and examine.—*C. Randall.*

LILLIE, R. D. **Studies on the decalcification of bone.** *Amer. J. Path.*, **20**, 291–6. 1944.

A variety of decalcifying agents were experimentally tested for their ability to decalcify bone and still permit staining of the blood elements with Romanovsky stains. It was found that satisfactory Romanovsky stains could be secured in decorticated cancellous bone decalcified with NH_4NO_3, NH_4Cl or KH_2PO_4, all of which had an initial pH of 5.0 or slightly above. Similar good results could be obtained on corticate bone by decalcifying with mixtures of sodium citrate and formic acid buffered to an initial pH above 3.0. Mixtures of formic acid and sodium citrate with a pH below 3.0 did not give as good results; these were buffered with mixtures of citric acid and K_2HPO_4.—*J. O. Foley.*

MOORE, MARGARET E. **Twenty-hour schedule for routine tissue sections.** *Canad. J. Med. Techn.*, **2**, 70–1. 1940.

To speed up results where 24-hour laboratory service is available, the author recommends the technic here given. Keep tissues from 3 P.M. until 8 P.M. in modified Dubascq-Brasie fixative prepared as follows: formaldehyde, 80 ml.; acetic acid, 40 ml.; normal saline, 250 ml.; alcohol (used), 400 ml.; picric acid, 4 g. Run through four changes of methanol in 7 hr., 1 hr. in acetone, 2 hr. in each of two changes of toluene, 3 hr. in each of two baths of paraffin, and embed. Cut sections, adhere to slide with 1% aqueous gelatin, and dry in oven. Treat with xylene, alcohol, and water. Stain 5 min. with Mayer's acid hemalum prepared as follows: distilled water, 1000 ml.; hematoxylin, 1.5 g.; $NaIO_3$, 0.2 g.; potassium alum, 50 g.; chloral hydrate, 50 g.; citric acid, 1 g.; mix and let dissolve at room temperature. Place in running water until blue. Counterstain with 0.1% aqueous eosin 3–4 min. Wash in tap water, dehydrate in alcohol, clear in carbol xylene and xylene, mount in Canada balsam.—*C. Randall.*

PAUL, PAULINE, LOWE, B., and McCLURG, B. R. **Changes in histological structure and palatability of beef during storage.** *Food Research*, **9**, 221–33. 1944.

The histological changes during the onset and dissolution of rigor and subsequent ripening of beef were investigated by the following technic: Slides were

made of both the raw and the cooked meat. The tissues were fixed in 10% formalin. Both paraffin and frozen sections were prepared. The paraffin sections were stained with French's modification of Weigert's elastic tissue stain, Harris' alum hematoxylin and van Gieson's mixture of acid fuchsin and picric acid. From the paraffin treatment the muscle fibers were stained yellow-orange, the collagenous tissue bright red, and the elastic tissue dark blue-green. The frozen sections were stained with Harris' alum hematoxylin and Herxheimer's "scarlet R" solution, giving blue muscle tissue and red fat deposits.

Rigor was shown histologically by the formation of dense nodes of contracture bordered on either side in the same fiber by areas of extreme stretch. Fibers which did not contract were drawn into waves and kinks by the shortening produced by the contracted fibers. Normal rigor produced denser nodes than those caused by heat when cooking of the beef was started before the onset of rigor.

The passing of rigor and progress of ripening were indicated by the appearance of breaks in the fibers. Sharp fractures usually occurred in the passively retracted fibers and granular or disintegrated areas in the stretched portions of the fibers adjacent to the rigor nodes.

The tests described were made on only one animal. Twenty pictures are given including a number of photomicrographs.—*William G. Walter.*

PINKUS, HERMANN. **Acid orcein Giemsa stain (modification of Unna-Taenzer method).** A useful routine stain for dermatologic sections. *Arch. Dermat. & Syph.*, **49**, 355–6. 1944.

For dermatological staining the author combines acid orcein (1 g. orcein in 100 ml. 70% ethyl alcohol, with 0.6 ml. concentrated HCl; usable immediately, but improving on standing) with dilute Giemsa solution (1 drop of "any good Giemsa stock solution" to 20 ml. distilled water). All the staining is done in jars, and the procedure is as follows: Deparaffinize the sections in xylene; transfer to 95% alcohol; 70% alcohol; stain 30–60 min. with acid orcein solution; rinse 2 min., or more in distilled water; wipe off excess water and dip briefly into 95% alcohol; decolorize 5–30 min. in absolute alcohol. (The sections should now be pale brown; the elastic fibers should stand out deep purple or black when observed under low power.) Decolorize in acid absolute alcohol (one drop conc. HCl to 10 ml.) until the background is almost colorless. (This step increases contrast considerably, but it should not be extended over 2–10 min. lest thin elastic fibers become decolorized.) Transfer to tap water for 5 min., or longer. Stain 2–12 hr. with dilute Giemsa solution. (Epithelial and other cells now should be deep blue and connective tissue grayish pink, grayish blue or fairly deep blue.) Wipe off excess fluid and dehydrate in 95% alcohol containing a few drops of 1% alcoholic eosin Y, e.g. the alcohol used for dehydration of hematoxylin and eosin sections. (The tissues lose their blue tinge fairly rapidly in this step, and decolorization must be stopped when the connective tissue has just lost all trace of blue and has become faintly rose. The epidermis should remain bright blue.) Cover with absolute alcohol, 2 changes, for 2 min. each; xylene, 2 changes; mount in Canada balsam.

A well stained section shows the following contrasts: Nuclei, deep blue; the cytoplasm of the epidermis, muscle cells and connective tissue cells, light blue; plasma cells, dark grayish blue; eosinophilic granules, bright red; mast cell granules, metachromatic purple; neutrophilic granules, usually not well stained; red corpuscles, brownish red; collagenous fibers, light pink, possibly with a faint brownish tinge; elastic fibers, dark brown or black; senile degenerated connective tissue (collacin, elacin and collastin), presenting various combinations of black, gray and blue; cartilage, matachromatic purple; decalcified bone, light brown; keratohyalin, blue (usually not prominently stained); stratum lucidum, dark rose; the keratin layer, colorless, light blue or light pink, depending on the tissue reaction and the degree of decolorization; the inner root sheath of the hair, deep blue; melanin granules, green or greenish black; other pigment, particularly hemosiderin, unstained; bacteria and mycelia, deep blue; *Demodex folliculorum*, in hair follicles, brown with blue granulations.—*J. A. Kennedy.*

PUGSLEY, MARION L. **Reticulocyte staining.** *Canad. J. Med. Techn.*, **3**, 16–7. 1940.

The following procedure is recommended: Prepare aqueous stain by dissolving 1 g. brilliant cresyl blue in 100 ml. 0.2% potassium oxalate. Mix one drop of stain with one drop of blood in small wax block. Blend by gentle blowing; allow to stand 1 min. Make smears and stain with any of the Romanovsky stains. Counterstaining with Wright's or Leishman's facilitates the examination of the smears, but reticulocytes may be counted with no further staining.

This method has three great advantages: the reticulum is heavier and thus more easily identified, the cells are not distorted, and the use of a centrifuge is obviated.—*C. Randall.*

SLAVKIN, ALICE E. **Quick paraffin method for small biopsies.** *J. Lab. and Clin. Med.*, **29**, 74. 1944.

The following method is useful in the microscopic examination of fragments of tissue too small for frozen sections: Fix in 4% formalin or Zenker's fluid for 10 min. at room temperature. Stain in any hematoxylin for 1 min. Wash in tap water. Dehydrate in 80%, 95% and absolute alcohols each 3 min. Clear in xylene 5 min. Transfer to melted paraffin, 2 changes for 5 min. each at 56° C. Embed in paraffin. Cut thin sections, transfer to slides and dry for 5 to 10 min. at 50° C., cool and stain as usual. Sections may be obtained within an hour.—*John T. Myers.*

PLANT MICROTECHNIC

STUART, NEIL W., and EMSWELLER, S. L. **Use of enzymes to improve cytological techniques.** *Science*, **98**, 569–70. 1943.

This is a preliminary note on the use of enzymes, particularly Clarase, a proprietary enzyme complex, to overcome clumping of chromosomes in the first meiotic metaphase of *Lilium*. Buds were fixed in a solution composed of three parts absolute alcohol to one part glacial acetic acid, then run through 95% alcohol to 70% alcohol for storage. For enzyme treatment the fixed buds were run through lower strengths of alcohol to water and thoroughly washed, then treated with the enzyme solution. With Clarase, treatment of 15 to 20 min. was as effective as several hours; definite reduction of chromosome clumping was obtained. Treatment of buds with several other types of solutions did not yield positive results.—*T. M. McMillion.*

WITTLAKE, EUGENE B. **Permanent prestaining in botanical microtechnic.** *Ohio J. of Sci.*, **44**, 36–8. 1944.

The author presents a method of staining blue-grass leaves in which both the primary stain and the counterstain are up to standard as to brilliance and intensity. The procedure is as follows:

Cut leaves transversely into one-eighth inch lengths. Place material in formal-acetic-alcohol (70% ethyl alcohol) or Bouin's fluid, and by means of a vacuum pump, apply a vaccum of 600 mm. mercury for 12 hr. Rinse quickly in distilled water. Transfer to 48% commercial HF for 120 hr. Remove and wash 1 hr. in running tap water. Transfer to Johansen's 50% tertiary butyl alcohol and pump 1 hr. at 600 mm. mercury. Stain 96–120 hr. in safranin Y made according to Johansen's procedure of preparing safranin O with methocellosolve. Dehydrate by Johansen's tertiary butyl alcohol method up to 100% at 2 hr. intervals. Transfer at 2 hr. intervals to 10, 25, 50, 70, 85, 95, 100% toluene series made up in tertiary butyl alcohol. Transfer to equal parts of xylene and toluene, and finally to 100% xylene at 2 hr. intervals. Place in embedding oven for 30 min. To infiltrate, add rubber paraffin to xylene at 3 hr. intervals and pour off one-half volume of mixture until odor of xylene is removed. Section. Remove paraffin with xylene. Agitate slide 45 sec. in equal parts of xylene and absolute alcohol, then in absolute alcohol for 45 sec. Counterstain 30–45 sec. in Johansen's fast green FCF. Agitate 1 min. in Johansen's fast green rinse (1 part xylene; 1 part clove oil; 1 part 100% ethyl alcohol). Clear 2 min. in synthetic methyl salicylate. Complete in 2 changes of xylene at 2 min. intervals. Mount in xylene, balsam, or clarite.—*C. Randall.*

MICROÖRGANISMS

ALEXANDER-JACKSON, ELEANOR. **A differential triple stain for demonstrating and studying non-acid-fast forms of the tubercle bacillus in sputum, tissue and body fluids.** *Science*, **99**, 307–8. 1944.

The following procedure is a modification of the Ziehl-Neelsen technic for demonstrating non-acid-fast forms of the tubercle bacillus: prepare smears which are not too thick and stain 3 min. with carbol-fuchsin; decolorize 1 to 3 min. with acid alcohol (3% HCl) and wash thoroughly in running water; flood slides with a well ripened Loeffler's methylene blue; then add 6 to 8 drops of freshly prepared *N* NaOH, letting stand for not more than 1 min.; flood the slides one at a time with sodium hydrosulfite solution, prepared by adding a "pinch" of sodium hydrosulfite to 50 ml. of tap water, to decolorize all except red acid-fast and blue non-acid-fast tubercle bacilli; wash off quickly in running water and flood the slide with an aqueous solution of equal volumes of 1% acid green and 1% acid yellow; wash off the stain and blot dry at once. This triple method of staining should be a useful supplement to the usual Ziehl-Neelsen technic since it reveals a number of interesting non-acid-fast forms which ordinarily escape observation.—*T. M. McMillion.*

BARRITT, M. M. **An improved Pappenheim stain for gonococci.** *Brit. Med. J.*, **494**, 4344. 1944.

Suggestions to overcome some of the criticisms of the author's procedure and the variability in the dyes are given together with a modified formula: methyl green, chloroform treated, 0.1–0.5%, 100 ml.; absolute methanol, 10 ml.; pyronin G, 0.5 g. or more; phenol, 1 g.; and glycerol, 20 ml.—*H. Leverne Williams.* (Courtesy *Biological Abstracts*).

BARTHOLOMEW, J. W., and UMBREIT, W. W. **Ribonucleic acid and the Gram stain.** *J. Bact.*, **47**, 415. 1944.

Pure crystalline ribonuclease rendered Gram-positive organisms Gram-negative under conditions in which the bacteria remained Gram-positive in the absence of the enzyme.— *V. Kavanagh.*

BAUER, WILLIAM H. **Tooth buds and jaws in patients with congenital syphilis.** *Amer. J. Path.*, **20**, 297–319. 1944.

The author reports superior results with an adaptation of the silver technic of Bertarelli and Volpino, a modified Levaditi method for staining *Treponema pallidum* in bone. The tissues are fixed in 2% formaldehyde or alcohol, decalcified in 5% HNO_3, blocked and placed in 5% $Na_2SO_4 \cdot 10\ H_2O$ for 2 days. Subsequently they are washed in tap water for 2 days and placed in a freshly prepared acidified alcoholic solution of $AgNO_3$ ($AgNO_3$ 1.5 g., distilled water 50 ml., 96% alcohol 50 ml., glacial acetic acid 4 to 5 drops). The blocks of tissue remain in the silver bath at 37° C., in the dark, for 18 days. The silver solution is changed when it becomes cloudy. Following impregnation with silver the tissues are washed for 1 day in frequent changes of distilled water. After washing, the blocks of tissue are reduced (tannic acid 3 g., gallic acid 5 g., sodium acetate 10 g., distilled water 350 ml.) in the dark, at room temperature, for 48 hours. The reducing solution is changed when it becomes cloudy. Following reduction, the tissues are washed thoroughly in distilled water, dehydrated and embedded in celloidin or paraffin. If paraffin is used, cedar oil is interposed between xylene and paraffin. The organisms stain black; the tissue yellow. The sectioned tissues may be subsequently counterstained with hematoxylin and eosin if desired.—*J. O. Foley.*

BEGG, A. M., FULTON, F., and VAN DEN ENDE, M. **Inclusion bodies in association with typhus rickettsiae.** *J. Path. & Bact.*, **56**, 109–13. 1944.

Rickettsiae in mouse and rabbit lung are readily demonstrated by the following modified Macchiavello method: Make thin impression smears of the cut surface of infected lungs. Fix by heat. Just before use mix 9 parts of 0.5% aqueous basic fuchsin solution with 1 part of M/15 phosphate buffer at pH 7.6, and filter

onto the slide. After 5 min. wash off the buffered fuchsin solution with tap water, and differentiate for 30–90 sec. with M/50 citrate buffer at pH 3.0. Wash with tap water. Counterstain for 30 sec. with 1% aqueous methylene blue. The rickettsiae are red and the cells blue. The results are illustrated with color photomicrographs.—*S. H. Hutner.*

CHEREWICK, W. J. **Studies on the biology of Erysiphe graminis DC.** *Canad. J. Research*, **22**, 52–85. 1944.

This is a study of the distribution and prevalence in Canada, the method of overwintering, the physiologic specialization, and the effects of various environmental factors on the development of the fungus *Erysiphe graminis*. To determine whether the conidia were uninucleate, they were dusted onto a slide lightly smeared with egg albumin. The slide was then flooded with aceto-carmine and a small crystal of hematoxylin was added; the slide was placed in a moist chamber at ordinary room temperature for 18 to 24 hr. The nuclei were well stained. Equally good results were obtained if the slide was warmed slightly for 4–6 hr.—*H. P. Riley.*

DISSMANN, EDWIN. **Erfahrungen mit der karbolnachtblaufärbung der Tuberkelbazillen nach Hallberg.** *Zentbl. Bakt., I Abt. Orig.*, **150**, 268–75. 1943.

The author suggests the following technic as an improvement over the classic Ziehl-Neelsen method for staining tubercule bacilli. Cover air-dried and flamed smear with night blue solution prepared in the following manner: dissolve 5 g. night blue (Grübler) in 100 ml. 95% alcohol (*solution A*); add 0.2 ml. 10% KOH to 100 ml. distilled water and then add 2.5 ml. liquid phenol (*solution B*); combine 1 part solution A with 10 parts solution B immediately before use. Slowly warm to boiling, cool 5 min., pour off solution. Destain in a mixture of 3 ml. 25% HCl and 100 ml. 70% alcohol until all possible blue has been removed. Wash with distilled water. Counterstain 5–10 sec. with 2% aqueous Bismark brown. Wash with distilled water and dry. (As alternate counterstains, the author recommends an acetic neutral red, a carbol pyronin, or a diluted carbol fuchsin.)

It was found that this technic has both qualitative and quantitative advantages. The tubercule bacilli were more darkly stained. Granules, often hard to recognize, appeared dark blue against the fine, pale blue stroma. In addition, when similar smears were stained by the Ziehl-Neelsen technic and with night blue, the latter method gave a larger bacterial count.—*C. Randall.*

HUNT, GEORGE A. **A study of the Pappenheim stain. A stable modification.** *J. Lab. & Clin. Med.*, **29**, 207–10. 1944.

The following formula is suggested: Mix 1.2 ml. of 1% aqueous malachite green (oxalate, certified "NMg 3", dye content 75%), 3.0 ml. of 0.5% aqueous pyronin G (certified "NP-7", dye content high) or pyronin 2 G (Coleman and Bell), and 20 ml. of distilled water. Prepare a stock solution of the malachite green 2 days before use and filter. Apply the stain for 30 sec. to 3 min. and wash with water. The stain is of special value in the examination of blood specimens, heavy smears, and blood clot cultures of chancroidal pus. Ducrey's bacillus stains much more distinctly than by the Gram technic.—*John T. Myers.*

KIRSH, DAVID, and SCHENKEN, JOHN R. **A comparison of the Ziehl-Neelsen and the Moss cold carbol fuchsin stains for acid-fast bacilli.** *New Orleans Med. and Surg. J.*, **96**, 394–6. 1944.

In contrast to the conventional Ziehl-Neelsen stain for acid-fast bacilli, the Moss cold carbol fuchsin method consists in fixing the smears in commercial formalin for 8 minutes, rinsing in water, and then staining with cold carbol fuchsin for about 4 minutes at room temperature. The advantage of this method is the avoidance of steaming the slide and the resultant saving of time. The Moss cold carbol fuchsin method gave as satisfactory stains as the Ziehl-Neelsen method.—*Robert Schrek.* (Courtesy *Biological Abstracts*).

LEE, H. I. **Comparison of procedures for staining tubercle bacilli in fluorescent microscopy.** *J. Lab. & Clin. Med.*, **29**, 218–21. 1944.

As compared with a number of alternatives, the following is the best procedure: Cover the smear for 5 min. with 0.1% auramine containing 5% phenol. Wash with tap water. Decolorize with 25% concentrated H_2SO_4 in 70% alcohol. Counterstain with 1:1000 aqueous $KMnO_4$ which overcomes interfering fluorescence. Staining with methylene blue is unnecessary.—*John T. Myers.*

NOBLE, GLENN A. **A five-minute method for staining fecal smears.** *Science*, **100**, 37–8. 1944.

A rapid procedure, well adapted to staining protozoa in fecal smears, is proposed: With a brush make a very thin smear of the material to be examined; cover smear immediately with an iron alum solution [formalin 10% by vol., 3 parts; glacial acetic acid, 1 part; $FeNH_4(SO_4)_2 \cdot 12H_2O$, 3 g. per 100 ml. of formol-acid mix]; pass slide through a flame until the fixative begins to steam; add more solution to the slide to prevent drying and quickly pour off the mordant fixative; immediately add several drops of 0.5% aqueous hematoxylin and pass slide through flame one or two times, the fecal material becoming a dark purple in 3 or 4 seconds; wash in running tap water in a Coplin jar 1 min.; remove all excess water from the slide with blotting paper and transfer the slide to dioxane; put the slide in a second jar of dioxane, 1 min.; transfer to a solution of dioxane and toluene, half and half, 30 sec. or more; mount in clarite.—*T. M. McMillion.*

HISTOCHEMISTRY

DISCHE, ZACHARIAS. **Two characteristic and sensitive color reactions between sulfhydryl compounds and thymonucleic acid.** *Proc. Soc. Exp. Biol. & Med.*, **55**, 217–8. 1944.

Thymonucleic acid and cysteine react with each other in H_2SO_4 at 40° with the formation of pink or reddish-brown end products, probably due to the formation of some furfuryl compound from desoxyribose. Cysteine in $1:10^6$ and thymonucleic acid in $1:10^4$ can be detected. Glutathione, 1:300,000, also reacts. The character of the reaction varies depending upon which of the two reactants is in excess.—*M. S. Marshall.*

WILMER, HARRY A. **Failure to demonstrate alkaline phosphatase activity in inclusion bodies by the histochemical technic.** *Proc. Soc. Exp. Biol. & Med.*, **55**, 206–7. 1944.

Alkaline phosphatase activity was not demonstrated in inclusion bodies of vaccinia, herpes simplex, fowlpox, or tracheo-laryngitis. The technic, modified from Gomori's method, was based on staining for insoluble calcium phosphate precipitated at the site of enzymic activity. Alcohol-fixed sections were incubated in a solution of sodium-β-glycerophosphate and $CaCl_2$ at the proper pH.—*M. S. Marshall.*

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